

CONFIDENTIAL

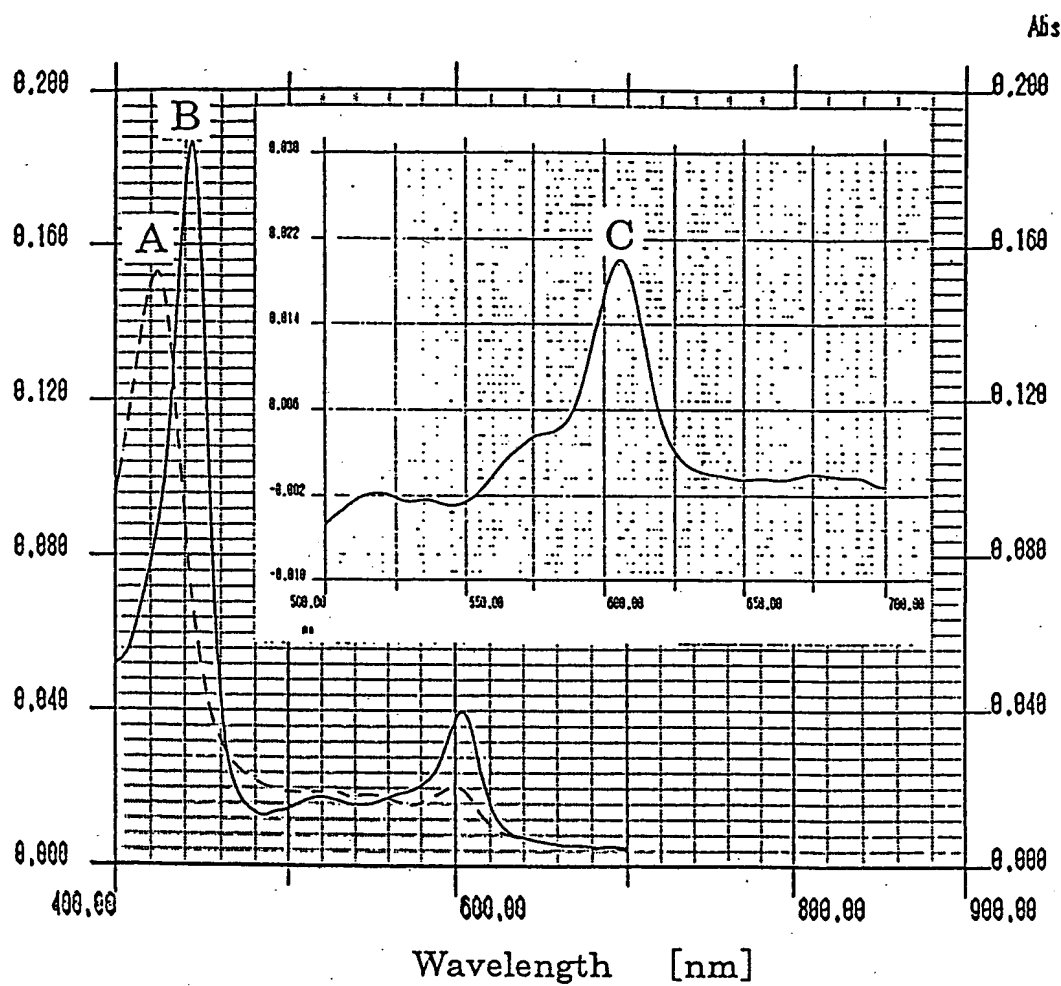


Figure 1

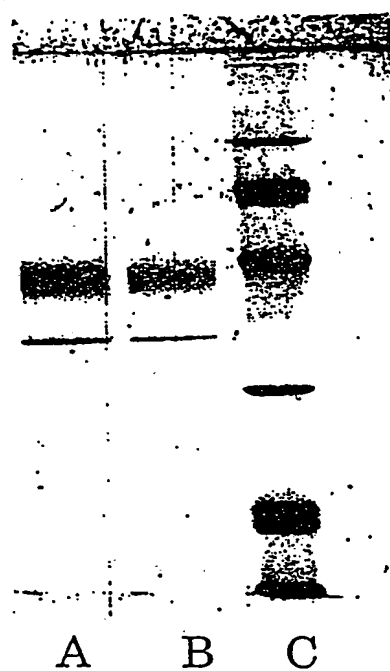


Figure 2

CO I

groups of 10

G.O./peptide

KDIGLLYLVAAGVVG

P.D. 1:MS-AQISDSIEEKGFFTRWFMSTNHDIGVLYLFTAGLAGLISVTLTVYMRMELQHPGVQYMC---LE-
R.S. 1:MADAAIHGHEHRRGFFTRWFMSTNHDIGVLYLFTGGLVGLISVAFVYMRMELMAPGVQFMCAEHLES
B.M. 1:-----M-FINRWLFSTNHDIGTLYLLFGAWAGMVG---TA-LSL-L----IR---AE-L--

P.D. 71:GM-R-LVAD---AAA-E-CTPNAHLWNVVVY-HGILMMFFVVI PALFGGFGNYFMPLHIGAPDMAFPRLN
R.S. 71:GLVKGFFQSLWPSAVENCTPNGLWNVMI-YGHGILMMFFVVI PALFGGFGNYFMPLHIGAPDMAFPRMN
B.M. 71:GQ-PG---TL---LGD---D-QIYNVVVT-AHAFVMIFFMVMPIMIGGFGNWLPLMIGAPDMAFPRMN

P.D. 141:NLSYWLVCVSLAIASLLSPGGSDQPGAGVGWVLYPPL-ST-TEAGYAMDIAIFAVHVSATSILGAIN
R.S. 141:NLSYWLVCVSLAVASLAPGGNGQLGSGIGWVLYPPL-ST-SESGYSTDLAIFAVHLSGASSILGAIN
B.M. 141:NMSFWLLPPSFLLLASSMVEAG---AGT---GWTVPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAIN

P.D. 211:IITFTFLNMRAPGMTLFKVPPLFAWVFI TAWMILLSLPVLAGGITMLLMDRNFQTFDPAAGGDPVLYQH
R.S. 211:MITFTFLNMRAPGMTLHKVPLFAWSIFVTAWLILLALPVLGAITMLLTDRNFQTFDPAAGGDPVLYQH
B.M. 211:FITTIINMKPPAMSQYQTPFVWSVMITAVLLLSLPVLAAGITMLLTDRNLNTFFDPAAGGDPVLYQH

G.O./PCR

WFFGHPEVYIIILPGFGIISHVSTFS-KKPVFGYLPVYAMLAI GVLGFVVAHMM

>>>>>

<<<<<

P.D. 281:ILWFFGHPEVYMLILPGFGIISHVISTF-ARKPIFGYLPVYAMLAI GVLGFVVAHMMYTAGMSLTQQT
R.S. 281:ILWFFGHPEVYIIVLPAFGIVSHVIATF-ARKPIFGYLPVYAMLAI GVLGFVVAHMMYTAGMSLTQQS
B.M. 281:LFWFFGHPEVYIILPGFGMISHIVTYYSCKKEPFGYMGVWAMMSIGFLGFIVVAHMMFTVGMVDVTRA

P.D. 351:YFQMATMTIAVPTGIKVFWSIATMGGSIIEFKTPMLWALA--FLFTVGGVTGVVIAQGSILDRVYHDTYYI
R.S. 351:YFMATMTIAVPTGIKVFWSIATMGGSIIEFKTPMLWALGFLFLFTVGGVTGIVLSQASVDRYHDTYYV
B.M. 351:YFTSATMTIAIPTGVKVFWSLATLHGGNIKWSPAMMIALGFI FLFTVGGLTGIVLANSLSLIVLHDTYYV

P.D. 421:VAHFHYVMSLGAIFAIFAGTYYSIGKMSGRQYPE-WAGQLHFWMFIFGNSLIFFPQHFLGRQGMPPRYID
R.S. 421:VAHFHYVMSLGAIFAGTSGIGKMSGRQYPE-WAGKLHFWMFIFGANLIFFPQHFLGRQGMPPRYID
B.M. 421:VAHFHYVLSMGAVFAIMGGFVHWPLFSGYTLNDTWA-KIHFAIMFVGVMNTFFPQHFLGLSGMPPRYS

P.D. 491:YPVEFSYWMNISSIGAYISFASFLFFIGIVFYTLFAGKPVNPNYWNHADTLEWTLSPPPERTFETLP
R.S. 491:YPEAFATWNVSSLGAFLSFASFLFLGVIFYSL-SGARVTANNYWNHADTLEWTLSPPPERTFEQLP
B.M. 491:YPDATWNTISSMGSFISLTAVMLMV-FIIWEAFASKR-EVLTV-DLTTTNLENLNGCPPPYHTFEE-P

P.D. 561:KPEDWDRAQAHR
R.S. 561:KREDWERAPAH-
B.M. 561:TYVN-LK-----

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria).
>>>>> <<<<< : amino acid sequences for PCR primers (Fig. 6)
G.O./peptide: amino acid sequence from the purified enzyme
G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 3

COII

P.D. 1:MAIATKRRGVAAVMSLG VATMTAVPALAQDVLGDL PVIGKPVNGGMNFQPASSPLAHDQQWLDHFVLYII
R.S. 1:MRHSTTLTPCATGAA-GLLAATAAAA-QQOTL-E--IIGRPQPGGTGFHGSASPVATQIHWLDGFILVII
B.M. 1:-----MAYPM-Q--LG-FQDATSPIM--EELLHFHD-HT---L-MIVF-L--I

G.O./peptide KASQETHNTPLEIVVTIVFV
G.O./PCR QETHNTPLEIVVTIVFVVLVFIGAFSLPVLFRQQEFPE-GDI
>>>>>

P.D. 71:TAVTIFVCLLLICIVRNRANFVPARFTHNTPIEVITLVPVLILVAIGAFSLPILFRSQEMPNDPDL
R.S. 71:GAITIFVTLILYAVWRFHKRNKVPARFTHNSPLEIAWTIVPILVAIGAFSLPVLFNQQEIP-EADE
B.M. 71:SSLVLYIISLIL--TTKLTHSTNDP----QE--VETIWTILPAIILILIALPSLRILYMMDEI-NNPSL

G.O./PCR VINVEGRSWYWGYE
<<<<<<

P.D. 141:VIKAIGHQWYWSYEYPNDAFDALML-----E-K-----E-ALADAGYSEDEYLLATDNFVVVPVG
R.S. 141:TVKVTGYQWYWGYYEPDEEISFESYMGSPATGGDNRM-SPEVEQQLEAGYTRDEFLLATDTAMVVPVN
B.M. 141:TVKTMGHQWYWSYEY----TDY-EDL--S--L-D-SYM-IPTSE--L-KPG---ELRLLEVDNRVVLPM

P.D. 211:KKVLVQVTATDVIHAWTIPAFVAVKQDAVFGRIAQLWFSVDQEGVYFGQCSELCGINHAYMPIVVKAVSQE
R.S. 211:KTVVVQVTGADVHSWTVP-FGVRQDAVFGRIAQLWFAEREGIFFGQCSELCGISHAYMPIVVKVSEE
B.M. 211:MTIRMLVSSGDVLHSAVPSLGLKTDAPGRINQTTLMSSRPGLYYGQCSEICGSNHSFMPIVLELVPLK

P.D. 281:KYEAWLAGAKEEFAADASDYLPAFPVKLASAE
R.S. 281:AYAAWLEQARGG-TYELSSVLPATPAGV-SVE
B.M. 281:YFEKW-SASML-----

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria).
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G.O./peptide: amino acid sequence from the purified enzyme
G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 4

CO III

P.D. 1:MAHVKNHDYQILPPSIWPFPGAIGAFVMLTGAVAWKGITFFGLPVEGPMFLIGLVGVLYVMFGWADV
R.S. 1:MAHAKNHDYHILPPSIWPFMASVGAFVMLNGAVLWMH-----G-S--GPWMGLIGLVVLYTMFGWSDV
B.M. 1:MTH-QTHAYHMVNPSPWPLTGALSALLMTSGLTMW---FHFNMTL----L-MIGLTTNNLTMVQWWRDV

P.D. 71:VNE-GETGEHTFVVRIGLQYGFILFIMSEVMFFVANFWAFIKNALYPMGPDSPIKDGVMPPEGIVTFDPW
R.S. 71:VTE-SLEGDHTFVVRIGLQYGFILFIMSEVIFFSANFWSFFKHALYPMGPESPIIDGIFPPEGIITFDPW
B.M. 71:IRESTFQGHHTPAVQKGLRYGMILFIISEVLFFTGFFWAFYHSSL---AP-TPELGCCWPPTGIHPLNPL

G.O./PCR TWAHHA-IVHGDRKRTAIGLALAIAGLGWIFTLCQAYEYIEIVHTE:xxxxxx
>>>>>>

P.D. 141:HLPLINTLILLSGVAVTWAHAFVLEGDRKTTINGLIVAVILGVCFTGLQAYEYSHAAGLADTVYAG
R.S. 141:HLPLINTLILLSCGAATWAHHALVHENNRDVAWGLALALALGALFTVFAQAYEYSHAAGFAGTIYGA
B.M. 141:EVPLLNTSVLLASGVSTWAHHS-LMEGDRKHMQLALFITITLGVYFTLLQASEYIEAPFTISDGVYGS

G.O./PCR xxxxxxxxxxxxxxxD-SIFLLVCLIRILRGAMSAKHVGFEMAANYWHFV

P.D. 211:AFYMATGFHGAHVIIIGTIFLFLVCLIRLLKGAMTQKHVGFEEAANYWHFVDVWVWLFVVIYIWR
R.S. 211:NFFMATGFHGFHVIVGTIFLLVCLIRVQRGHFTPEKHVGFEEAANYWHFVDVWVWLFVVIYIWR
B.M. 211:TFFVATGFHGLHVIIGSTFLIVCFRQLKFHFTSNHFGFEAGAWYWHFVDVWVWLFVVIYIWR

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*;
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>>>>>> <<<<<< : amino acid sequences for PCR primers (Fig. 6)

G.O./PCR: amino acid sequence deduced from DNA amplified by PCR (PCR/DNA)

Figure 5

CO I (target size: approx. 180 bp DNA)

A: W F F G H P → *

5'-TGGTTCTTCGGNCACCC-3'

T T T

B: ← V W A H H M *

3'-CANACCCGNGTAGTATAC-5'

G G

CO II (target size: approx. 180 bp DNA)

A: Q F T H N T → **

5'-CAATTTACNCATAATAC-3'

G C C C

B: ← W Y W G Y E Y *

3'-ACCATAACCCCNATACTTAT-5'

G G C

CO III (target size: approx. 300 bp DNA)

A: T W A H H A → *

5'-CANTGGGCNCATCATGC-3'

C C

B: ← W Y W H F V D *

3'-ACCATAACCGTAAAACANCT-5'

G G G

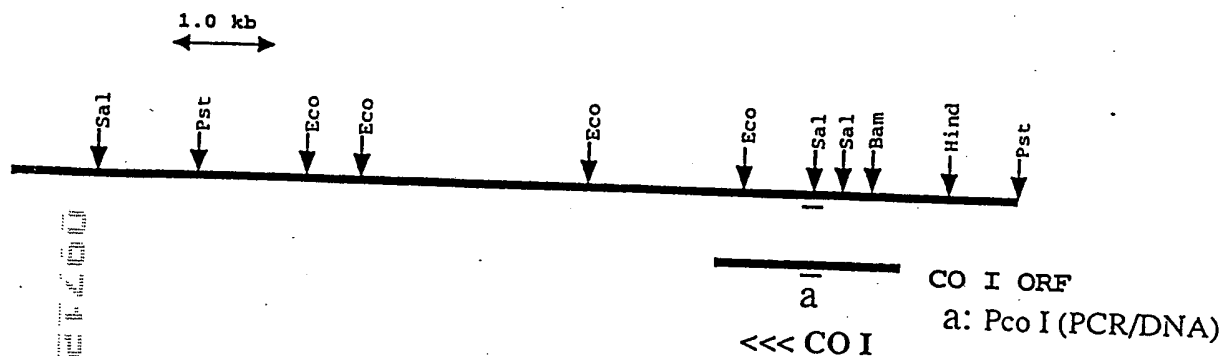
N : A, T, G, C

* : based on consensus sequence

** : based on the peptide sequence

Figure 6

8.0 kb *Pst*I fragment containing CO I ORF of pUCO01



9.3 kb *Eco*RI fragment containing CO II and III ORFs of pUCO23

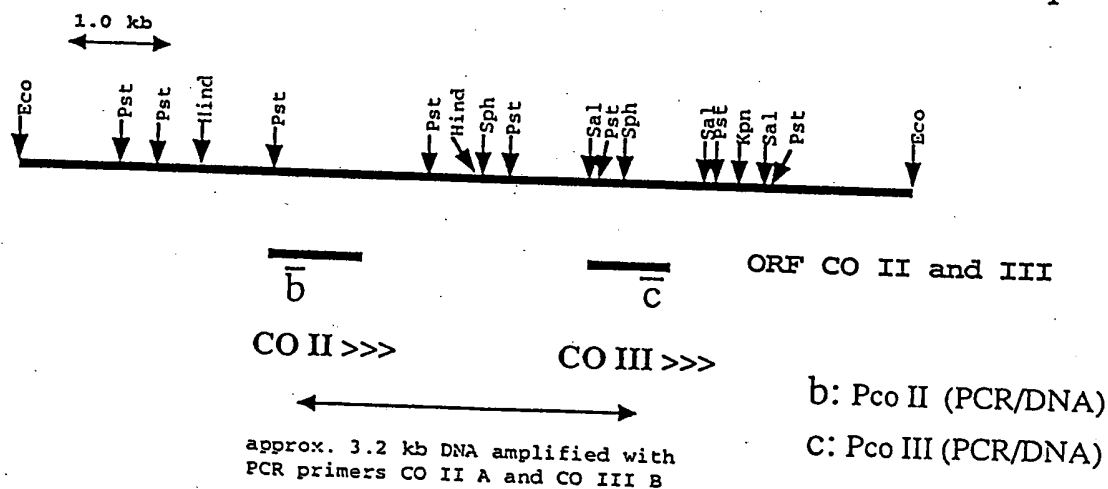


Figure 7

00013361200

B.M. : -----M-FINRWLFSTNHKDIGTLYLLFGAWAGMVGTALESLLIRAELEGPG--TL----L-----G--D-
P.D. : MS-AQI-SDSIEEKGFFTRWFMSTNHKDIGVLYLFTAGLAGLISVTLTVYMRMELQHPGVQYMC---LE-GM-R-LV-A
R.S. : MADAAIHGHE-HDRRGFFTRWFMSTNHKDIGVLYLFTGGLVGLISVAFVYMRMELMAGVQFMCAEHLESGLVKGFQFS
G.O. : MADAAIHGHDHHEKQGFTRWFMSTNHKDIGLLYLVAAGVVGFI SVLFTVYMRLELMDPGVQYMC---LE-G-AR--L--

B.M. : -----D-QIYNVVVT-AHAFVMIFFMVMPIMIGGFGNWLVLPMIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
P.D. : ---DAAAECTPNAHLWNVVVY-HGILMMFFVVI PALFGGFGNYFPLHIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
R.S. : LWPSAVENCTPNGLHWNVMI-YGHGILMMFFVVI PALFGGFGNYFPLHIGAPDMAFPRMNMNSFWLLPPSFLLLASSM
G.O. : I-ADASQTCTANGHLWNVMI-YGHGILMMFFVGI PALFGGFGNYLMLQIGAPDMAFPRMNMNSFWLLPPSFLLLASSM

B.M. : VEAG---AGT---GWTVPPLAGNLAHAGASVDLTIFSLHLGAVSSILGAINFITTI INMKPPAMSQVQTPLFVWSVMITA
P.D. : SPCGSDQPGAGVGVLYPPL-ST-TEAGYAMDLAIFAVHVSAGTSILGAINITITFLNMRAPGMTLFKVPFLFAWAVFITA
R.S. : APGGNGQLGSGIGVLYPPL-ST-SESGYSTDLAIFAVHLSGASSILGAINMITTFLNMRAPGMTLHKVPLFAWSIFVTA
G.O. : APGGDQQLGSGGVLYPPL-ST-REAGYSMDLAIFAVHLSGASSIMGAINMITTFLNMRAPGMTLHKVPLFSWIFITA

B.M. : VLLLLSLPVLAAITMLLTDRNLNTTFFDPAGGGDPILYQHLFWFFGHPEVYIILPGFGMISHIVTYYSKKKEPFGYMG
P.D. : WMILLSLPVLAGGITMLLMDRNFQTFDPAGGGDPVLYQHILWFFGHPEVYMLILPGFGIISHVISTF-ARKPIFGYLP
R.S. : WLILLALPVLAGAITMLLTDRNFQTFDPAGGGDPVLYQHILWFFGHPEVYIIVLPAFGIVSHVIATF-AKKPIFGYLP
G.O. : WLILLALPVLAGAITMLLTDRNFQTFDPAGGGDPILYQHILWFFGHPEVYIILPGFGIISHVVSTF-SKKPVFGYLP

B.M. : MVAMMSIGFLGFIVWAHMMFTVGMVDVTRAYTSATMIIAIPITGVKVSFLATLHGNGNIKWSPAMMHALGFIFLFTVGG
P.D. : MVLAMAAIAFLGFIVWAHMMFTAGMSLTQOTYFQMATMTIAVPTGIKVFWSIATMWGGSIEFKTPMLWA--LAFVFTVGG
R.S. : MVAMVAIGVLFVVAHMMFTAGLSLTQSSYFMMATMTIAVPTGIKVFWSIATMWGGSIEFKTPMLWALGFLFTVGG
G.O. : MVAMVAIGVLFVVAHMMFTVGMSTLQSSYFMLATMTIAVPTGIKVFWSIATMWGGSVEFKSPMLWAFGFMFLFTVGG

B.M. : LTGIVLANSSLDIVLHDTYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLNDTWA-KIHFAIMFVGVNMTFFPQHFL
P.D. : VTGVVIAQGS�DRVYHDTYVVAHFHYVMSLGAIFAIFAGTYYSIGKMSG-RQYPEWAGQLHFWMMFISNLIFFPQHFL
R.S. : VTGIVLSQASVDYHDTYVVAHFHYVMSLGAIFAIFAGTSGIGKMSG-RQYPEWAGKLHFWMMFVGANLTFFPQHFL
G.O. : VTGIVLAQAGLDYHDTYVVAHFHYVMSLGAIFAIFAGTYFYMFKFSG-RAPEWAAKLHFWTFFIGANVTFFPQHFL

B.M. : GLSGMPRRYSDYDAYTMWNTISSMGSFISLTAVM-L--MVFIWEAFASKREVLTV-DLTTNLEWLNGCPPPHTFEE
P.D. : GRQGMPPRYIDYVPEFSYWNMISSIGAYISFASLFFIGIVF-YTLFAGKPVNVPNYWNEHADTLEWTLSPPPHTFET
R.S. : GRQGMPPRYIDYPEAFATWNVSSLSGAFLSFASLFFLVIF-YSL-SGARVTANNYWNEHADTLEWTLSPPPHTFET
G.O. : GRQGMPPRYIDYPEAFALWNKVSSYGAFLAFASLFFFI-VIFVYTLVAGRRETRPNPWGEFADTLEWTLSPPPHTFET

B.M. : -PTYVN-LK-----
P.D. : LPKPEDWDRAQHR
R.S. : LPKREDWERAPAH-
G.O. : LPKRSWDKHPSH-

Homology among amino acid sequences of the CO I subunits of aa3-type cytochrome c oxidase

	B.M.	P.D.	R.S.
P.D.	52.2	-	-
R.S.	50.6	76.7	-
G.O.	53.3	76.0	78.7

[% homology]

Abbreviations: G.O. *G. oxydans* DSM4025; P.D. *Paracoccus denitrificans*; R.S. *Rhodobacter sphaeroides*; B.M. Bovine (Mitochondria)

Figure 8

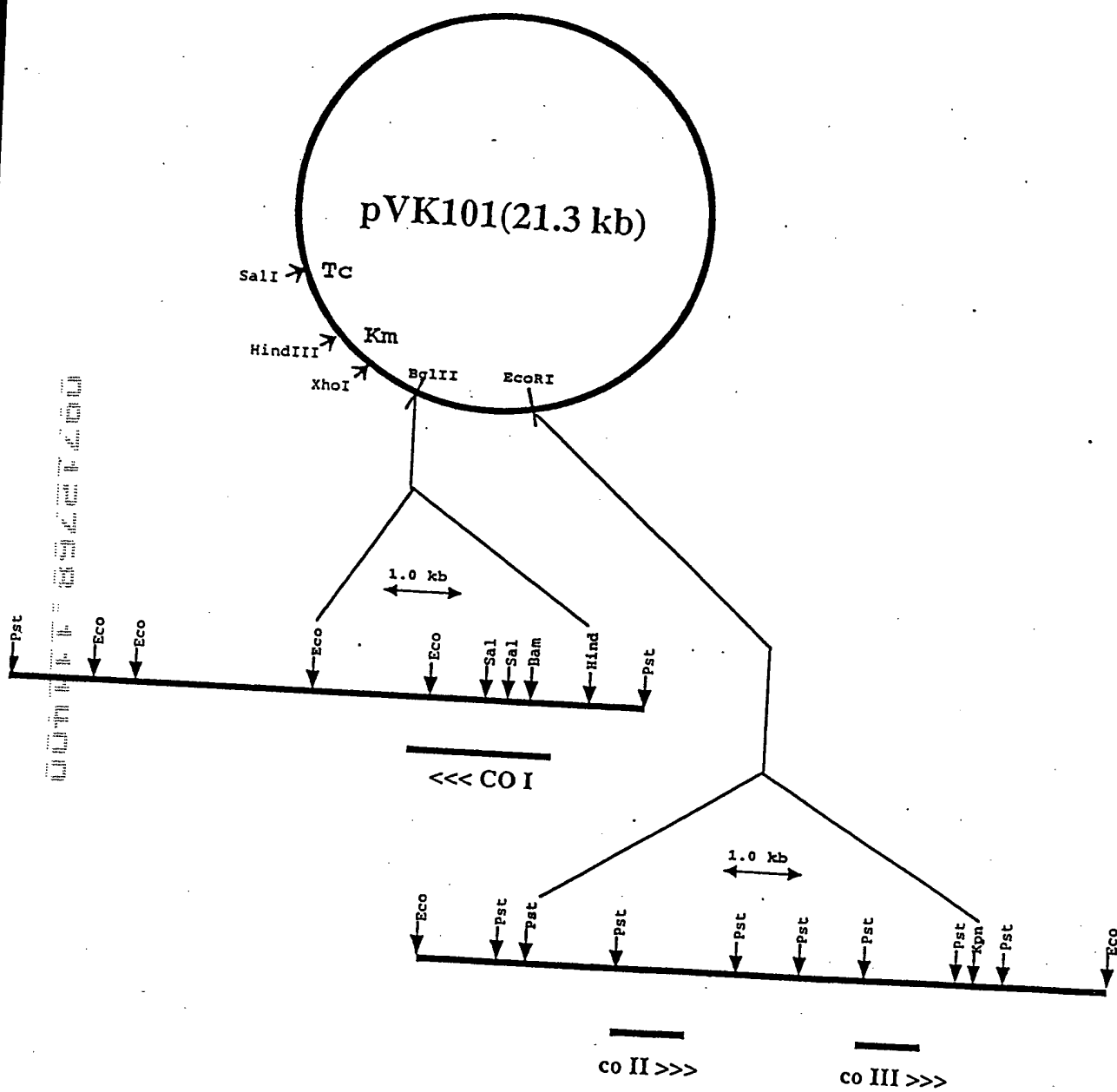


Figure 9